

available at [www.sciencedirect.com](http://www.sciencedirect.com)journal homepage: [www.intl.elsevierhealth.com/journals/arob](http://www.intl.elsevierhealth.com/journals/arob)

# A histomorphometric study of alveolar bone modelling and remodelling in mice fed a boron-deficient diet

Alejandro A. Gorustovich<sup>a,b,1,\*</sup>, Tammy Steimetz<sup>c,1</sup>,  
Forrest H. Nielsen<sup>d</sup>, María B. Guglielmotti<sup>b,c</sup>

<sup>a</sup> Research Laboratory, National Atomic Energy Commission (CNEA-Regional Noroeste), A4408FTV Salta, Argentina

<sup>b</sup> National Research Council (CONICET) C1033AAJ, Argentina

<sup>c</sup> Department of Oral Pathology, School of Dentistry, University of Buenos Aires, C1122AAH Buenos Aires, Argentina

<sup>d</sup> United States Department of Agriculture, Agricultural Research Service, USDA, ARS Grand Forks Human Nutrition Research Center, Grand Forks 58202-9034, ND, United States

## ARTICLE INFO

### Article history:

Accepted 19 January 2008

### Keywords:

Boron

Alveolar bone

Histomorphometry

Mice

## ABSTRACT

**Objective:** Emerging evidence indicates that boron (B) plays a role in bone formation and maintenance. Thus, a study was performed to determine whether dietary B-deficiency affects periodontal alveolar bone modelling and remodelling.

**Design:** Weanling Swiss mice ( $n = 30$ ) were divided into three groups: control diet (GI, 3 mg B/kg); B-deficient diet (GII, 0.07 mg B/kg); and pair-fed with GII (GIII). The animals were maintained on their respective diets for 9 weeks and then sacrificed. The guidelines of the NIH for the care and use of laboratory animals were observed. The mandibles were resected, fixed, decalcified in 10% EDTA and embedded in paraffin. Buccolingually oriented sections were obtained at the level of the mesial root of the first lower molar and stained with H-E. Histomorphometric studies were performed separately on the buccal and lingual sides of the periodontal alveolar bone. Percentages of osteoblast surfaces (ObSs), eroded surfaces (ESs), and quiescent surfaces (QSs) were determined.

**Results:** No statistically significant differences in food intake and body weight were observed between the groups. When compared with GI and GIII mice, GII mice (B-deficient) had 63% and 48% reductions in ObS and 58% and 73% increases in QS in buccal and lingual plates, respectively. ES were not affected by B nutriture.

**Conclusion:** The results are evidence that dietary boron deprivation in mice alters periodontal alveolar bone modelling and remodelling by inhibiting bone formation.

© 2008 Elsevier Ltd. All rights reserved.

## 1. Introduction

Alveolar bone is the most malleable of the periodontal tissues, because it is subjected to continuous modelling and remodelling associated with tooth eruption and functional require-

ments.<sup>1–3</sup> Furthermore, the environment influences healthy and diseased periodontal tissues.<sup>3,4</sup> It has been stated that diet and nutrition are major multifactorial environmental factors in the etiology and pathogenesis of craniofacial disorders, i.e. periodontal diseases.<sup>5–7</sup>

\* Corresponding author at: Research Laboratory, National Atomic Energy Commission (CNEA-Regional Noroeste), A4408FTV Salta, Argentina. Tel.: +54 387 4254000; fax: +54 387 4250502.

E-mail address: [agorustovich@gmail.com](mailto:agorustovich@gmail.com) (A.A. Gorustovich).

<sup>1</sup> These authors contribute equally to this work.  
0003-9969/\$ – see front matter © 2008 Elsevier Ltd. All rights reserved.  
doi:10.1016/j.archoralbio.2008.01.011

Nutrition is an important modifiable factor in the development and maintenance of bone mass. Dietary components, such as protein, vitamins, and trace elements are required for normal bone metabolism.<sup>8,9</sup> Emerging evidence indicates that boron (B) plays a role in bone formation and maintenance.<sup>10–14</sup> To the best of our knowledge, the role of dietary B on alveolar bone modelling and remodelling has not been addressed. Thus, the aim of the present study was to perform a histological and histomorphometric evaluation of periodontal alveolar bone modelling and remodelling under a B-deficient diet in mice.

## 2. Materials and methods

### 2.1. Animals

Thirty male weaned (21 d old) Swiss mice were used throughout. They were housed in steel-cages and maintained on a 12:12 h light–dark cycle. All animal experiments were carried out in keeping with the guidelines of the National Institutes of Health for the care and use of laboratory animals (NIH Publication No. 85-23, Rev. 1985). The protocol was examined and approved by the Institutional Ethics Committee of the School of Dentistry, University of Buenos Aires.

### 2.2. Experimental design

The animals were assigned to 1 of 3 groups, with each group containing 10 animals: control diet (GI, 3 mg B/kg); B-deficient diet (GII, 0.07 mg B/kg); and pair-fed with GII (GIII). The basal diet (Table 1) similar to that used in other studies<sup>10,15</sup> was based on ground corn that was acid-washed<sup>16</sup> to reduce its boron content, and vitamin-free casein. It contained adequate amounts of all known essential nutrients plus some mineral elements (e.g., nickel, silicon, vanadium) in nutritional quantities that have been found beneficial to bone health.<sup>17</sup>

Fresh powder diet and deionized water in plastic cups were provided *ad libitum*. Body weight and food intake were determined. The animals were maintained on their respective diets for 9 weeks and then sacrificed. The mandibles were resected and fixed in 10% formalin solution.

### 2.3. Histological processing

The mandibles were decalcified in 10% EDTA and embedded in paraffin. Buccolingually oriented sections were obtained at the level of the mesial root of the first lower molar and stained with hematoxylin–eosin.

### 2.4. Histological and histomorphometric evaluation

Histological studies and histomorphometric measurements were performed separately on the buccal and lingual sides of the periodontal alveolar bone, which correspond to remodelling and modelling activities, respectively. To clearly define the sides it was necessary to establish the apical limit of the alveolus.<sup>18,19</sup> Within this context a line *a* was drawn tangent to the upper cortical of the mandibular canal. Another line was drawn between the uppermost points (A and B) of the buccal

**Table 1 – Composition of the basal diet<sup>a</sup>**

Ingredient	g/kg
Ground corn, acid-washed	713.486
Casein, vitamin-free	160.000
Safflower oil	75.000
Tert-butylhydroquinone	0.014
DL- $\alpha$ -Tocopherol	0.200
Choline chloride	1.000
L-Cystine	2.000
Vitamin mix <sup>b</sup>	4.000
Macro-mineral mix <sup>c</sup>	29.300
Trace mineral mix <sup>d</sup>	15.000
Total	1000.0

<sup>a</sup> Analyzed concentration of boron was about 0.07 mg (9  $\mu$ mol)/kg. To make a 3 mg boron/kg diet, a mix containing 0.0172 g H<sub>3</sub>BO<sub>3</sub> and 0.9828 g dextrose replaced 1.0 g of ground corn in the basal diet.

<sup>b</sup> Composition of the vitamin mix (in mg): vitamin A palmitate (500,000 IU/g), 16; thiamine HCL, 10; pyridoxine HCL, 15; nicotinic acid, 30; DL-pantothenic acid, 48; vitamin B<sub>12</sub> (0.1% in mannitol), 50; folic acid, 2; biotin, 1; riboflavin, 27; vitamin K (phyloquinone), 1; inositol, 50; para aminobenzoic acid, 5; vitamin D<sub>3</sub> (400,000 IU/g), 2.5; and dextrose, 3,742.5.

<sup>c</sup> Composition of the macro-mineral mix (in g): CaHPO<sub>4</sub>, 17.0; KCl, 7.0; and Mg(C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>)<sub>2</sub>·4H<sub>2</sub>O, 5.3.

<sup>d</sup> Composition of the trace element mix (in mg): NaCl, 2000; Mn(C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>)<sub>2</sub>·4H<sub>2</sub>O, 45; CuSO<sub>4</sub>·5H<sub>2</sub>O, 30; Zn(C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>)<sub>2</sub>·2H<sub>2</sub>O, 84; iron powder (dissolved in HCl), 75; NaHAs<sub>4</sub>·7H<sub>2</sub>O, 5; KI, 0.4; NaSeO<sub>3</sub>·5H<sub>2</sub>O, 1.4; Cr(C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>)<sub>3</sub>·2H<sub>2</sub>O, 2; NH<sub>4</sub>VO<sub>3</sub>, 0.3; (NH<sub>4</sub>)<sub>2</sub>MoO<sub>4</sub>, 1; NaF, 2; NiCl<sub>6</sub>H<sub>2</sub>O, 3.7; NaSiO<sub>2</sub>·9H<sub>2</sub>O, 50, and ground corn (acid-washed), 12700.2.

and lingual crests. Line CD was drawn so that it bisected the distance between A and B and was perpendicular to line *a*. In this way the buccal side was limited by points A and D, and the lingual side by points B and D (Fig. 1). The following parameters were determined: percentage of osteoblast surface (ObS), eroded surface (ES), and quiescent surface (QS). Osteoblast surfaces are covered by osteoid seams and mature osteoblasts. Eroded surfaces are scalloped by Howship's lacunae with or without osteoclasts. Quiescent surfaces are covered by bone lining cells. Histomorphometric evaluation was performed using a microcomputer-based image analysis system (Kontron Elektronik Company, Munich, Germany).

### 2.5. Statistical analysis

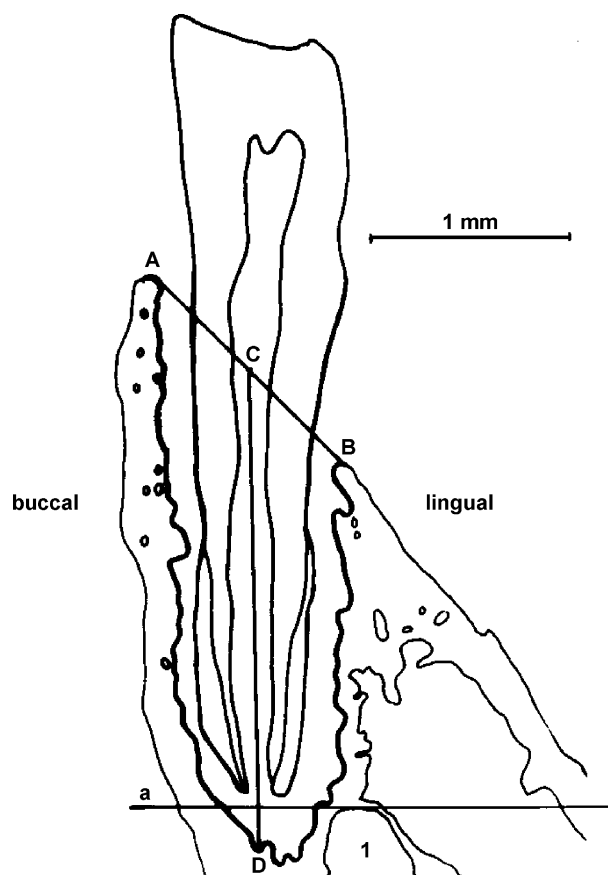
The values for each treatment group were presented as mean  $\pm$  standard deviation.

The statistical significance of the data was determined using the analysis of variance test (ANOVA). When ANOVA showed a significant difference, the Newman–Keul's multiple-range test was used to define the differences ( $P < 0.01$ ) among groups.

## 3. Results

### 3.1. Body weight and food intake parameters

No statistically significant differences in food intake and body weight were observed between groups (data not shown).



**Fig. 1 – The buccal side of the periodontal alveolar bone was considered to lay between points A and D; the lingual side was considered to lay between points B and D. 1: mandibular canal.**

### 3.2. Histological and histomorphometric findings

#### 3.2.1. Buccal plates

Light microscopy observation revealed, for GI animals, a predominance ( $52 \pm 9\%$ ) of bone surface lined by mature cuboidal osteoblasts (ObS) and bone lining cells (QS) ( $48 \pm 9\%$ ).  $2 \pm 3\%$  of the bone surface was lined by ESs (Figs. 2A and 4A).

In comparison to group I, group II animals exhibited a statistically significant reduction ( $63\%$ ,  $P < 0.01$ ) in the percentage of osteoblast surfaces (ObSs,  $19 \pm 10\%$ ) concomitantly with an increase ( $58\%$ ,  $P < 0.01$ ) in quiescent surfaces (QSs,  $76 \pm 11\%$ ). No statistically significant differences was observed for eroded surfaces as compared to GI and GIII animals (Figs. 2B and 4A).

Group III animals did not show any statistically significant differences with GI animals for any of the parameters evaluated (Fig. 4A).

#### 3.2.2. Lingual plates

The ObS ( $60 \pm 5\%$ ) predominated in GI animals. QS comprised  $40 \pm 7\%$  of the total (Figs. 3A and 4B).

Group II animals exhibited a statistically significant reduction ( $48\%$ ,  $P < 0.01$ ) in ObS ( $31 \pm 7\%$ ) and a statistically significant increase ( $73\%$ ,  $P < 0.01$ ) in the surfaces lined by

bone lining cells (QS,  $69 \pm 7\%$ ) as compared with group I (Figs. 3B and 4B).

Group III animals did not show any statistically significant differences with GI animals for any of the parameters evaluated (Fig. 4B).

None of the groups (I, II, III) exhibited eroded surfaces in the lingual plates.

## 4. Discussion

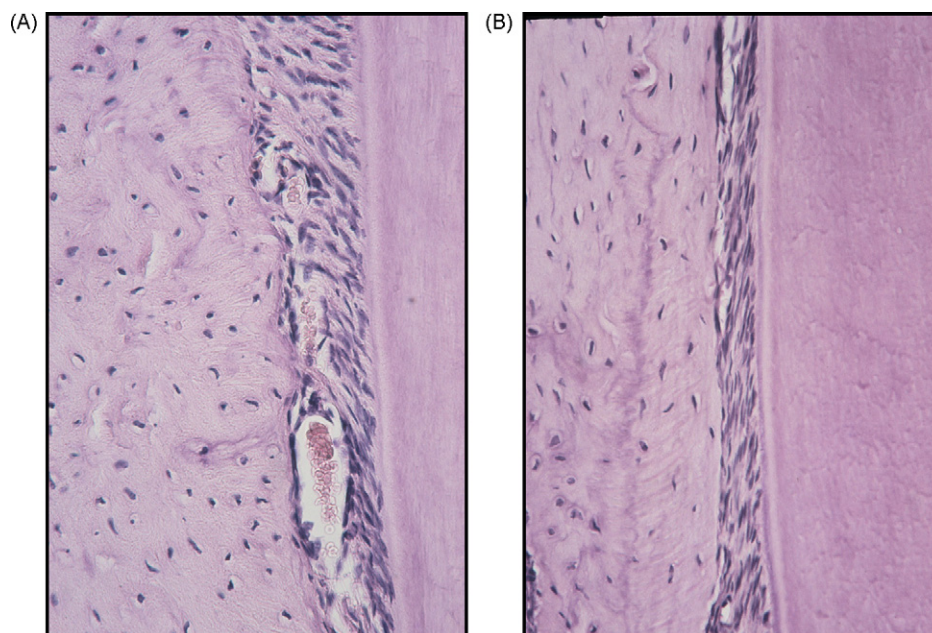
The present results provide, for the first time, evidence that the dietary boron (B) deficiency affects the alveolar bone. The histological and histomorphometric analysis evidenced an alteration in periodontal alveolar bone modelling and remodelling in B-deficient mice in terms of a reduction in osteogenic activity concomitantly with an increase in quiescent surfaces. Eroded surfaces were not affected by B nutriture. The differences between the buccal and lingual plates would be due to the differences in behaviour between plates, i.e. remodelling and modelling in the buccal and lingual plates, respectively, as reported previously.<sup>18,19</sup> After the eruption period, the relationship between the teeth and their supporting structures remains dynamic, as the former migrate spontaneously within the alveolar process. Teeth migrate mesially in humans and primates but bucco-distally in rodents.<sup>1,20,21</sup>

Alveolar bone is constantly renewed by modelling and remodelling mechanisms in response to functional demands, local and systemic factors.<sup>1,18–22</sup> Nutritional deficiencies in animals have been shown to affect the periodontal tissues.<sup>4–7,23–25</sup> In this study, we determined that dietary B deprivation alters periodontal alveolar bone modelling and remodelling by inhibiting bone formation.

Epidemiologic data do not support the suggestion that nutritional deficiencies play an important role in the aetiology and pathogenesis of periodontitis.<sup>4</sup> In addition, the efficacy of nutrient supplementation for the therapeutic modulation of the host response in the management of chronic inflammatory periodontal diseases, remains to be determined.<sup>7,26,27</sup> One of the most practical applications of nutritional modulation of chronic diseases may be nutrients that regulate the expression of key inflammatory genes.<sup>28–30</sup> It has been demonstrated that dietary B supplementation may down-regulate inflammation at a site upstream of cytokine gene activation in the NF- $\kappa$ B regulated pathway.<sup>31</sup> Further studies are necessary to evaluate the role of B in the inflammation associated with periodontal disease.

The present study reveals the importance of dietary B in mice periodontal health. The exact cellular and molecular mechanisms by which B deficiency affects alveolar bone remains to be elucidated.

The present findings are consistent with other findings indicating that B deprivation adversely affects bone formation and microstructure. In one study<sup>13</sup> the fourth lumbar vertebrae from male rats exposed to B deprivation (0.1 mg/kg diet) from conception to age 21 weeks were examined by microcomputed tomography and compared to vertebrae from rats fed supplemental B (3 mg/kg diet). Boron deprivation decreased bone volume fraction and trabecular thickness, and

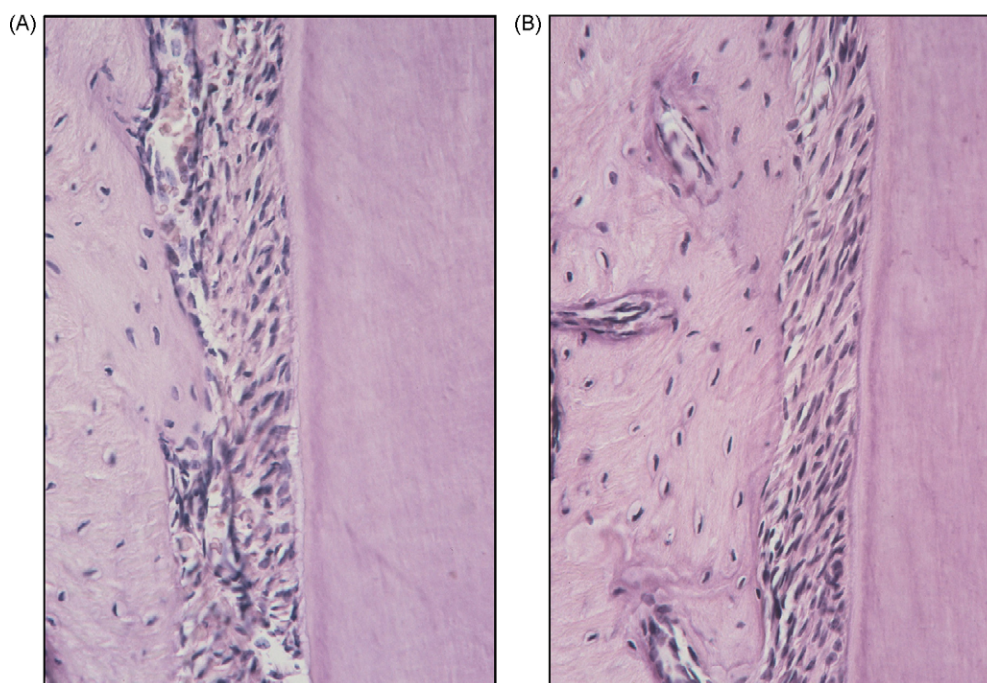


**Fig. 2 – Microphotograph of the buccal side of the periodontal alveolar bone. (A) Control diet (GI, 3 mg B/kg) for 9 weeks. Note the bone surface lined by mature osteoblasts. (B) B-deficient diet (GII, 0.07 mg B/kg) for 9 weeks. Note the bone surface lined by bone lining cells (hematoxylin–eosin stain; original magnification 400×).**

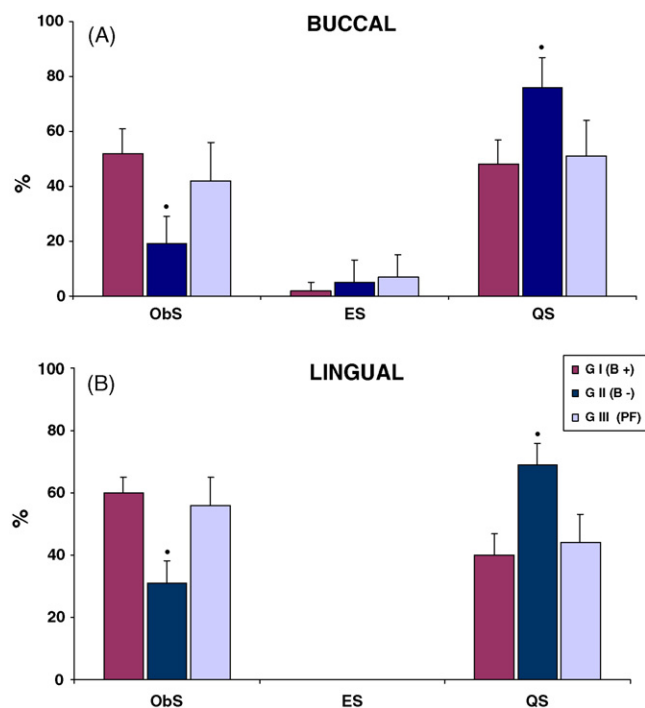
increased trabecular separation and structural model index (a lower value or more plate-like structure is preferable).

Interestingly, B deprivation does not markedly affect the calcium and phosphorus concentrations in bone. Instead, B deprivation affects the concentrations of mineral elements (e.g., magnesium, potassium, copper, zinc) <sup>10,13</sup> associated

with the formation, differentiation and activity of osteoblasts and osteoclasts. The mineral changes in bone, in addition to B deprivation decreasing alveolar bone osteoblast surface in rats<sup>14</sup> and mice (present study), and chondrocyte density in the growth plate of proliferation of chicks,<sup>32</sup> suggests that B is beneficial to bone growth and maintenance through affecting



**Fig. 3 – Microphotograph of the lingual side of the periodontal alveolar bone. (A) Control diet (GI, 3 mg B/kg) for 9 weeks. Note the bone surface lined by mature osteoblasts. (B) B-deficient diet (GII, 0.07 mg B/kg) for 9 weeks. Note the bone surface lined by bone lining cells (hematoxylin–eosin stain; original magnification 400×).**



**Fig. 4 – Histomorphometric study. (A) Buccal side of the periodontal alveolar bone. (B) Lingual side of the periodontal alveolar bone. Values are means  $\pm$  S.D.; (\*)  $P < 0.01$  compared with GI values. Percentages of osteoblast surfaces (Obs), eroded surfaces (ESs), and quiescent surfaces (QSs).**

osteoblast and/or osteoclast presence or activity and not through affecting bone calcium concentration.

In conclusion, our findings suggest that dietary B deprivation in mice alters periodontal alveolar bone modelling and remodelling due to an inhibition of bone formation.

## Acknowledgements

This study was supported by United States Department of Agriculture, Agriculture Research Service USDA, ARS Extramural Agreement 58-5450-4N-F038; and by Grants UBA 0020 and CONICET PIP 6042.

The authors wish to acknowledge the technical assistance of Alicia Araoz (Department of Oral Pathology, School of Dentistry, University of Buenos Aires), and Jim Lindlauf (USDA ARS, Grand Forks Human Nutrition Research Center) for animal diet preparation. We also thank Drs Edward C Carlson and Curtiss D Hunt (Department of Anatomy and Cell Biology, School of Medicine and Health Sciences, University of North Dakota) for critical reading of the manuscript and helpful suggestions.

## REFERENCES

- Lindhe J, Karring T, Araújo M. Anatomy of the periodontium. In: Lindhe J, Karring T, Lang NP, editors. *Clinical periodontology and implant dentistry*. 4th ed. Copenhagen: Blackwell Munksgaard; 2003. p. 3–49.
- Nanci A, Bosshardt DD. Structure of periodontal tissues in health and disease. *Periodontol* 2000;40:11–28.
- Bartold PM, Narayanan AS. Molecular and cell biology of healthy and diseased periodontal tissues. *Periodontol* 2000;40:29–49.
- Kinane DF, Peterson M, Stathopoulou PG. Environmental and other modifying factors of the periodontal diseases. *Periodontol* 2000;40:107–19.
- Position of the American Dietetic Association. Oral health and nutrition. *J Am Diet Assoc* 2003;103:615–25.
- Moynihan PJ. The role of diet and nutrition in the etiology and prevention of oral diseases. *Bull World Health Organ* 2005;83:694–9.
- Jepsen R, Kuchel GA. Nutrition and inflammation: the missing link between periodontal disease and systemic health in the frail elderly? *J Clin Periodontol* 2006;33:309–11.
- Prentice A, Schoenmakers I, Laskey MA, de Bono S, Ginty F, Goldberg GR. Nutrition and bone growth and development. *Proc Nutr Soc* 2006;65:348–60.
- Heaney RP. Bone health. *Am J Clin Nutr* 2007;85:300S–3S.
- Nielsen FH. Dietary fat composition modifies the effect of boron on bone characteristics and plasma lipids in rats. *Biofactors* 2004;20:161–71.
- Gallardo-Williams MT, Maronpot RR, Turner CH, Johnson CS, Harris MW, Jayo MJ, et al. Effects of boric acid supplementation on bone histomorphometry, metabolism, and biomechanical properties in aged female F-344 rats. *Biol Trace Element Res* 2003;93:155–69.
- Naghii MR, Torkaman G, Mofid M. Effects of boron and calcium supplementation on mechanical properties of bone in rats. *Biofactors* 2006;28:195–201.
- Nielsen FH, Stoecker BJ, Penland JG. Boron as a dietary factor for bone microarchitecture and central nervous system function. In: Xu F, Goldbach HE, Brown PH, Bell RW, Fujiwara T, Hunt CE, Goldberg S, Shi L, editors. *Advances in Plant and animal boron nutrition*. Dordrecht, The Netherlands: Springer; 2007. p. 277–90.
- Gorustovich AA, Steimetz T, Nielsen FH, Guglielmotti MB. A histomorphometric study of alveolar bone healing in rats fed a boron-deficient diet. *Anat Rec*; in press.
- Nielsen FH, Penland JG. Boron deprivation alters rat behavior and brain mineral composition differently when fish oil instead of safflower oil is the diet fat source. *Nutr Neurosci* 2006;9:105–12.
- Nielsen FH, Myron DR, Givand SH, Ollerich DA. Nickel deficiency and nickel–rhodium interaction in chicks. *J Nutr* 1975;105:1607–19.
- Nielsen FH. Boron, manganese, molybdenum, nickel, silicon, and vanadium. In: Driskell JA, Wolinsky I, editors. *Sports nutrition. Vitamins and trace elements*. Boca Raton: CRC Taylor & Francis; 2006. p. 287–320.
- Ubios AM, Guglielmotti MB, Steimetz T, Cabrini RL. Uranium inhibits bone formation in physiologic alveolar bone modeling and remodeling. *Environ Res* 1991;54:17–23.
- Gorustovich A, Steimetz T, Giglio MJ, Guglielmotti MB. A histomorphometric study of alveolar bone modeling and remodeling under experimental anaemia and polycythaemia in rats. *Arch Oral Biol* 2006;51:246–51.
- Saffar JL, Lasfargues JJ, Cherruau M. Alveolar bone and the alveolar process: the socket that is never stable. *Periodontol* 2000;13:76–90.
- Vignery A, Baron R. Dynamic histomorphometry of alveolar bone remodeling in the adult rat. *Anat Rec* 1980;196:191–200.
- Sodek J, McKee MD. Molecular and cellular biology of alveolar bone. *Periodontol* 2000;24:99–126.

23. Seto H, Toba Y, Takada Y, Kawakami H, Ohba H, Hama H, et al. Milk basic protein increases alveolar bone formation in rat experimental periodontitis. *J Periodont Res* 2007;**42**:85–9.
24. Orbak R, Kara C, Ozbek E, Tezel A, Demir T. Effects of zinc deficiency on oral and periodontal diseases in rats. *J Periodont Res* 2007;**42**:138–43.
25. Gorustovich A, Espósito MA, Guglielmotti MB, Giglio MJ. Mandibular bone remodeling under a choline-deficient diet: a histomorphometric study in rats. *J Periodontol* 2003;**74**: 831–7.
26. Neiva RF, Steigenga J, Al-Shammari KF, Wang HL. Effects of specific nutrients on periodontal disease onset, progression and treatment. *J Clin Periodontol* 2003;**30**:579–89.
27. Vardar-Sengul S, Buduneli N, Buduneli E, Kardesler L, Baylas H, Atilla G, et al. Dietary supplementation of omega-3 fatty acid and circulating levels of interleukin-1beta, osteocalcin, and C-reactive protein in rats. *J Periodontol* 2006;**77**:814–20.
28. Kornman KS, Martha PM, Duff GW. Genetic variations and inflammation: a practical nutrigenomics opportunity. *Nutrition* 2004;**20**:44–9.
29. Kornman KS. Interleukin 1 genetics, inflammatory mechanisms, and nutrigenetic opportunities to modulate diseases of aging. *Am J Clin Nutr* 2006;**83**:475S–83S.
30. Enwonwu CO, Ritchie CS. Nutrition and inflammatory markers. *J Am Dent Assoc* 2007;**138**:70–3.
31. Durick KA, Tomita M, Hunt C, Bradley D. Evidence that boron downregulates inflammation through the NF- $\kappa$ B pathway. *The FASEB J*. San Diego: The Federation of American Societies for Experimental Biology (FASEB), 2005;972:6 [Experimental Biology 2005 Meeting Abstracts]
32. Hunt CD, Herbel JL, Idso JP. Dietary boron modifies the effects of vitamin D<sub>3</sub> nutrition on indices of energy substrate and utilization and mineral metabolism in the chick. *J Bone Miner Res* 1994;**9**:171–82.